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Post-harvest N_2O and CO_2 emissions related to plant residue incorporation of oilseed rape and barley straw depend on soil NO_3- content

Sarah Köbke[a,*], Mehmet Senbayram[b,1], Birgit Pfeiffer[a,c], Heiko Nacke[c], Klaus Dittert[a]

[a] Department of Crop Science, Section of Plant Nutrition and Crop Physiology, University of Göttingen, Carl-Sprengel-Weg 1, 37075 Göttingen, Germany
[b] Institute of Applied Plant Nutrition, University of Göttingen, Carl-Sprengel-Weg 1, 37075 Göttingen, Germany
[c] Institute of Microbiology and Genetics, Department of Genomic and Applied Microbiology, University of Göttingen, Grisebachstr. 8, 37077 Göttingen, Germany

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ABSTRACT

The sustainable production of bioenergy from crops like oilseed rape, barley, and maize presents a significant option to mitigate climate change by reducing fossil CO_2 emissions. Greenhouse gas emissions (specifically N_2O) during the energy crop production need to be quantified precisely for reliable life cycle analysis of bioenergy cropping systems. Energy crops (specifically oilseed rape) have a very high N demand, which results in a higher N-fertilizer application and thus higher risk of N_2O emissions not only during the vegetation period but also after crop harvest due to i) incorporation of N rich plant residue to soil and/or ii) residual N. An incubation experiment was conducted under conditions favoring denitrification (80% water-holding capacity), to study the drivers of N_2O emissions specifically during the post-harvest period. Here we compared two different plant residues varying in C/N ratio (oilseed rape (RST) and barley straw (BST)) with or without N supply and measured CO_2, and N_2O emissions. Stable isotope labeling (^{15}N) was used to quantify soil- and residue-born N_2O. Incorporation of both plant residues alone induced significant increases in CO_2 emissions compared to control soil without straw addition ($p < .05$). However, the increase in CO_2 emissions was less pronounced when straw was incorporated in conjunction with mineral N. There was a clear increase in cumulative N_2O emissions ($p < .05$) when soil amended with BST or RST (6- and 9-fold) was compared to control, however, the increase of cumulative N_2O emissions was drastic when mineral N was added (15- and 23-fold). No significant differences in N_2O emission were observed when comparing residue types ($p > .05$). Stable isotope labeling of barley straw clearly showed that the share of residue-born N_2O was very low (1.35 or 0.4%) in the overall N_2O fluxes in BST and BST + N.

The present study suggests that N fertilization in autumn should be avoided to minimize N_2O fluxes regardless of type of straw.

1. Introduction

Renewable energies have gained great attention in policy making and the EU Renewable Energies Directive has been released aiming to increase the share of renewable sources in the energy supply to 20% (EU RED 2009/28/EC, 2009). The latter aims to reduce the consumption of fossil fuels and the emission of climate relevant carbon dioxide (CO_2). However, there is great concern that in the course of producing energy crops, the formation and emission of other potent greenhouse gases such as nitrous oxide (N_2O) would negate climate benefits. In northwestern Europe, oilseed rape based crop rotations have moved into focus as this crop has reached a large share in biodiesel production. Additional crops in oilseed rape crop rotations are the cereals barley and wheat. So far, greenhouse gas (GHG) emissions in oilseed rape cropping systems seem to be greater than in others, e.g. cereal rotations (Walter et al., 2014).

After harvest, straw incorporation is a common and important agricultural practice to improve soil fertility. It improves soil physical conditions like aggregate stability and water infiltration and chemical properties like pH as well as macro- and micro-nutrient availability (Blanco-Canqui and Lal, 2009). Added straw provides a source of organic carbon and energy, but also a small share of nitrogen for decomposing soil microorganisms, which are essential for C and N mineralization (Chen et al., 2014a) as well as for vital soil microbial communities. Several studies show that the incorporation of plant residues into the soil increases both, biomass and activity of soil

* Corresponding author.
E-mail address: sarah.koebke@agr.uni-goettingen.de (S. Köbke).
[1] Present address: Institute of Plant Nutrition and Soil Science, University of Harran, Osmanbey, 63000, Sanliurfa, Turkey.

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microorganisms (Blanco-Canqui and Lal, 2009; Potthoff et al., 2005; Rousk and Bååth, 2007), thus, contributing to CO_2 and also N_2O emissions from soil (e.g. Begum et al., 2014; Velthof et al., 2002). The velocity of CO_2 and N_2O emissions after straw incorporation depends on the mass and type of straw. Important characteristics are C and N concentrations of straw as well as the ratio of both (Huang et al., 2004). A low C/N ratio leads to the mobilization of straw C and N and its release into the soil mineral N pool which functions as substrates for microbial processes leading to CO_2 and N_2O emissions. Oilseed rape straw is ascribed a higher N concentration and a lower C/N ratio than barley straw which may accelerate its breakdown and its release of straw-N to the soil mineral N pool by soil microorganisms (Walter et al., 2014).

With respect to post-harvest processes in soil, there is another important post-harvest agricultural practice besides the incorporation of straw. Often N fertilizers are applied to foster straw mineralization and to warrant sufficient available N for the following crop. However, N addition to soil does not only promote residue mineralization and CO_2 production, but it may also favor nitrification and denitrification, potentially contributing to enhanced nitrate (NO_3^-) leaching and N_2O release from soil to atmosphere. This may pose economic and environmental risks (Crutzen, 1970; Kaiser and Brenninkmeijer, 2002; Prather and Hsu, 2010). Additionally, N_2O is one of the most potent greenhouse gases with a global warming potential being 265 times higher than that of CO_2 on a 100-year-basis (Myhre et al., 2013).

To prevent the release of large amounts of N_2O, it is crucial to distinguish the various chemical and biological pathways forming N_2O. The most dominant biological processes are nitrification, denitrification and also nitrifier denitrification (Wrage et al., 2001). The microbial formation of N_2O and N_2 is also enhanced by specific soil physiochemical properties. García-Marco et al. (2014) list various factors like high NO_3^- availability and a C source leading to favorable conditions for N_2O production by denitrification. The addition of a labile C source, e.g. straw or root exudates can increase microbial activities as well as greenhouse gas emissions. This effect is more pronounced if the added C-source has a low C/N ratio, as shown by Huang et al. 2004. Nevertheless, also the addition of straw with a high C/N ratio leads to increasing N_2O emissions, although they are lower than emissions from soils amended with low C/N straw (Huang et al. 2004).

A number of studies have highlighted the connection of organic amendments to agricultural soil and its importance on soil N cycling (e.g. Chen et al., 2013; Chen et al., 2014a; Knorr et al., 2005; Thangarajan et al., 2013). However, there is little information on the release of residue N in form of N_2O (Frimpong and Baggs, 2010; Gentile et al., 2008; Millar and Baggs, 2005). So far, there is evidence that the increase of released N_2O might not be directly triggered by N released from straw which entered the soil mineral N pool (Frimpong and Baggs, 2010; Millar and Baggs, 2005). In the course of agricultural management, plant residues are regularly incorporated into the soil after harvest where they influence nitrogen turnover processes.

In this context, we have following hypothesis:

(i) the annulment of N limitation will be followed by high soil CO_2 emissions and high soil mineral N content,
(ii) the low C/N ratio of the oilseed rape residue will lead to higher N_2O emissions compared to barley straw with high C/N, and
(iii) fertilizer N will promote the release of residue N as N_2O because of mineral N fluctuations that accelerate decomposition and soil N cycling.

For examining above mentioned hypothesis, we conducted an automated continuous flow incubation trial and monitored gas fluxes. In our incubation study, we focused on effects of incorporation of oilseed rape straw and barley straw on CO_2 emissions, soil N dynamics and N_2O emissions. In addition, N fertilizer application was considered to unravel potential nitrogen limitation of straw amended soils affecting the release of CO_2 and N_2O. Stable isotope labelling approach was used to study the share of straw-N emitted as N_2O. In addition, we quantified the genes encoding the subunit of the nitrous oxide reductase (*nosZ*), responsible for the reduction of N_2O to N_2.

2. Material and methods

2.1. Experimental incubation set-up

A soil incubation experiment was carried out in a fully automated continuous flow incubation system using 15 incubation vessels of 20 cm diameter and 22 cm height. Soil was repacked (5.8 kg FW, 4.8 kg DM) into each incubation vessel including control soils (non-treated soil) to a final soil density of 0.96 g soil DM cm^{-3}. The upper 15 cm of the mineral soil (Luvisol, clay 25%, silt 65.5%, sand 9.5%, pH $_{(CaCl2)}$ 6.6, C 1.02%, N 0.11%) had been collected in spring 2013 from an unfertilized farmer's field in Sattenhausen close to Goettingen (51.51° N, 10.13° E). It was carefully air dried to allow sieving with a 4 mm mesh sieve. Complete drying out was avoided to minimize mineralization after rewetting.

To simulate good agricultural practice, ^{15}N labeled barley (*Hordeum vulgare*, Total C: 41.17, Total N: 0.73, C/N: 56.45) or oilseed rape (*Brassica napus*, Total C: 43.97, Total N: 0.94, C/N: 46.61) straw was mixed with the upper 10 cm of soil prior to the experiment at a rate of 1.5 g straw DM kg^{-1} soil DM. The straw was cut with scissors to a length of 2 cm to avoid large straw particles.

Prior to the experiment, 0.325 g of N was applied to the soil surface in the form of calcium ammonium nitrate (CAN, solid commercial fertilizer, 100 kg N ha^{-1} equiv. to 67.5 mg N kg^{-1} soil) in the respective treatments following rewetting of soil to c. 80% water holding capacity (WHC) by carefully dripping distilled water on the soil surface including the control. WHC was determined by putting a soil column in a cylindrical tube with a water-permeable membrane fixed underneath. The tube with the soil column placed into a water bath to saturate the soil column with water. Subsequently, the tube with the soil column was removed from the water bath and left for 24 h so that excessive water could run off through the membrane by gravity. By weighing, water content left in the soil column can be calculated. The amount of water left after 24 h is equal to 100% WHC. To adjust to 80% WHC, the water contents of the fresh soil and that of 80% WHC were used for calculation. Moisture conditions normally vary from year to year. To simulate frequently occurring moist conditions in autumn and to favor denitrification, WHC has been set to 80%. N addition reflects a high soil N level as typical fertilizer rates range from 30 to 40 kg N ha^{-1} to agricultural fields in autumn.

All incubation vessels were sealed airtight and continuously flushed with synthetic air at a flow rate of 15 to 20 ml min^{-1} to ensure aerobic conditions. The experiment was carried out in a temperature controlled environment at 22 °C and lasted for 43 days. For additional soil sampling a parallel system was set up under the same conditions in the same laboratory. Soil sampling was done every other day and after day 10, the time between sampling of soil was increased. Soil samples for molecular analysis were collected at day 1, 7, 11 and 25 and stored at −80 °C until further use. Overall, there were five soil treatments including non-treated control soil (CK), barley straw incorporation only (BST), oilseed rape straw incorporation only (RST), barley straw + N (BST + N), or oilseed rape straw + N (RST + N), all carried out in three replications.

2.2. Soil analysis

For determination of soil mineral N content, 9 g of soil FW were sampled and immediately processed to minimize mineralization. Samples were extracted with a 0.0125 M $CaCl_2$ solution (1:5 w/v) for 45 min. on an overhead shaker (85 rpm). The extracts were filtered with 615 ¼ filter paper (Macherey – Nagel GmbH & Co. KG, Düren,

Germany) and stored at 20 °C. The extracts were analyzed colorimetrically for the concentrations of NO_3^- and NH_4^+ using the San + + Continuous-Flow Analyzer (Skalar Analytical B.V., Breda, The Netherlands). Soil water content was determined with a parallel set of samples.

2.3. Trace gas measurements

For online analysis of N_2O and CO_2, gas samples from all vessel outlets were directed sequentially to a Bruker gas chromatograph (450-GC, Bruker, Billerica, USA) via two multi-position valves (9 and 16 ports) with a multi-position actuator control module (Valco Instruments Co. Inc., Huston, TX, USA) and an interface module (506C System Interface, Gilson, Inc., Middleton, WI, USA) controlled by Trilution Software (Gilson Inc., Middleton, WI, USA). The gas samples were analyzed by GC deploying a thermal conductivity detector (TCD) for CO_2, and an electron capture detector (ECD) for N_2O. Gas samples were taken at least every 7 h for 23 days. The resolution in time was decreased to one analysis per day for the remaining period.

2.4. Stable isotope analysis ^{15}N-N_2O

For the stable isotope study in the incubation experiment, barley straw was labelled with ^{15}N and had an enrichment of 33.07 at% ^{15}N. Serum flasks were evacuated and filled with gas sample via the outlet pipe of each incubation vessel. The gas sample was injected by a two-hole-needle by flushing the serum flask with He as carrier gas. Before stable nitrogen isotope analysis of N_2O was carried out with an isotope ratio mass spectrometer (MAT 253, Thermo Scientific, Waltham, MA, USA), the collected gas sample was cleaned from water with a Nafion Tube and to separate compounds, a molsieve (5A) GC column was installed.

In order to obtain the share of ^{15}N labelled N_2O in the emitted N_2O, gas samples were taken every day in the first week, then with a 10-day gap and a final sampling after 5 more days. ^{15}N labelled barley straw has been produced by growing summer barley plants (*Hordeum vulgare*) in 30 L plastic containers in a screenhouse with 95 at% ^{15}N-labelled urea at a level of 460 mg N kg^{-1} soil DM. To calculate straw-N-derived N_2O, an equation according to Gentile et al. (2008) was used:

$$Q_{Straw} = Q_{Sample}\left(\frac{^{15}N_{Sample} - {}^{15}N_{natural\ abundance}}{^{15}N_{Straw} - {}^{15}N_{natural\ abundance}}\right)$$

where Q_{Straw} is the quantity of N in the N_2O-N emissions derived from the straw, Q_{Sample} is the N measured in the N_2O, $^{15}N_{Sample}$ is the at % of N_2O, $^{15}N_{natural\ abundance}$ is the natural abundance of the control soil and the $^{15}N_{Straw}$ is the at % of the straw.

The ^{15}N natural abundance of the control soils (average 0.369 at% ^{15}N) was measured on every sampling day and the mean was subtracted from the ^{15}N enrichment of the gas sample, respectively.

2.5. Extraction of RNA and cDNA synthesis

To quantify the transcription of bacterial *nosZ* genes encoding nitrous oxide reductase, total RNA was isolated from 1 g of soil per sample using the RNA PowerSoil™ total RNA isolation kit, as indicated by the manufacturer (MO BIO Laboratories Inc., Carlsbad, CA, USA). The resulting RNA pellet was dissolved in 50 µl RNase-free water. To avoid degradation of the isolated RNA, 0.7 µl of the RiboLock RNase Inhibitor (Thermo Scientific, Inc., Waltham, MA, USA) were added to each sample. Residual DNA contaminations were removed using the TURBO DNA-*free*™ kit (Life Technologies, Carlsbad, CA, USA) as described by the manufacturer. The DNA-free RNA was further checked for remaining DNA contaminations using PCR. Additionally, DNase-treated RNA was purified and concentrated in a final volume of 14 µl using the RNeasy MinElute cleanup kit (Qiagen GmbH, Hilden, Germany). The concentration of the purified RNA extracts was determined using the Qubit RNA HS Assay Kit (Life Technologies) and a Qubit fluorometer (Life Technologies). Subsequently, 0.5 µl RiboLock RNase Inhibitor (Thermo Scientific, Inc) were added to each sample to avoid degradation of the purified RNA extracts during storage at −80 °C and the following procedures. The cDNA synthesis was performed using the SuperScript™ II reverse transcriptase (Invitrogen, Karlsruhe). Initial denaturation and primer annealing was conducted in a mixture (12 µl) containing 10 µl of isolated DNA-free RNA, as well as 2 µM random primer pd(N)6 (Roche, Mannheim, Germany) and 10 mM dNTP mix, which was incubated for 5 min at 65 °C and chilled on ice for at least 2 min. A mixture (6 µl) containing 4 µl 5-fold First Strand buffer and 2 µl of 0.1 M DTT, was added. The reaction mixture was incubated for 2 min at 25 °C before adding 200 U SuperScriptTM II reverse transcriptase. The cDNA synthesis mixture was incubated for 10 min at 25 °C and for additional 50 min at 42 °C. The reaction was terminated at 70 °C for 15 min and the resulting cDNA was stored at −80 °C.

2.6. Quantification of nosZ gene transcripts

The *nosZ* standard used was derived from (Bannert et al., 2011). As standard, serial plasmid dilutions of the *nosZ* gene derived from *Pseudomonas fluorescence* using the primer pair 5′-CGCRACGGCAASAAGG-TSMSSGT-3′ (nosZ2F) and 5′-CAKRTGCAKSGCRTGGCAGAA-3′ (nosZ2R), were used (Henry et al., 2006). The final concentration of the plasmid extract was determined using the Qubit dsDNA BR Assay Kit (Life Technologies) and a Qubit fluorometer (Life Technologies). The copy number per µl was calculated based on the concentration, weight and length of the respective plasmids. The serial plasmid dilution ranged from 10^1 to 10^8 gene copies per µl. Quantification of *nosZ* gene transcripts was performed on an iQ™5 real-time PCR detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using the SYBR® Green PCR Master Mix (Life Technologies) and the same primer pair (nosZ2F and nosZ2R) used for standard preparations. To quantify the gene transcript copy numbers we used 1 µl of 1:10 diluted cDNA, equivalent to 0.5% of the extracted RNA per g soil. The reaction mixture (25µL) contained 12.5 µL SYBR® Green PCR Master Mix, 2 µM of each of the primers and the template DNA. The following thermal cycling scheme was used: initial denaturation at 95 °C for 15 min, followed by 46 cycles of denaturation at 95 °C for 15 s, annealing at 62 °C for 30 s, extension at 72 °C for 30 s and data acquisition step at 80 °C for 15 s. Data were acquired at 80 °C to avoid signals from non-specific products or primer dimers. Standard curves were generated to depict the relationship between gene copy numbers and threshold cycle values. Furthermore, standard curves were conducted on every 96-well plate in every qPCR run. Copy numbers of the analyzed soil samples were determined using the standard curves. All measurements were conducted in triplicate.

2.7. Statistics

CO_2 and N_2O emissions were shown as arithmetic mean of the three replicates with one standard error. The cumulative CO_2 and N_2O emissions were calculated by linear interpolation between measurements. Cumulative CO_2 emissions were analyzed with an ANOVA followed by a Tukey test. For cumulative N_2O emissions, a One Way ANOVA was performed with the focus on straw addition and excluding the + treatments. To check for pairwise multiple differences among groups, Tukey's test with $p < .05$ as criterion for significant differences was used. Statistical analyses were done with SigmaPlot 13.0 (Systat Software GmbH, Erkrath, Germany).

3. Results

3.1. Soil GHG emissions

Emissions of CO_2 from non-treated control soils increased

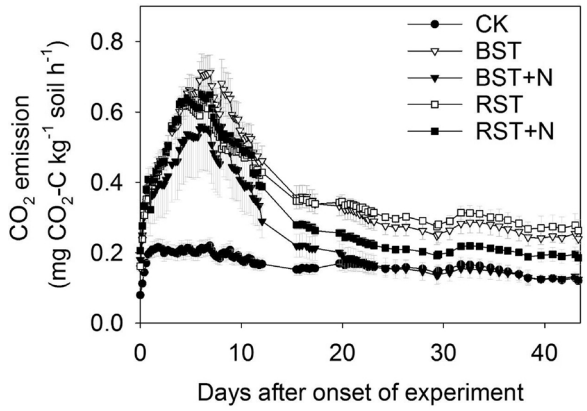

Fig. 1. Daily CO_2 emissions from soils after incorporation of oilseed rape straw (RST) and barley straw (BST) only and with N fertilizer (RST + N, BST + N) in the incubation experiment (CK: non-treated control soil). Error bars show the standard error of the mean of each treatment (n = 3). In some cases error bars are smaller than the symbols.

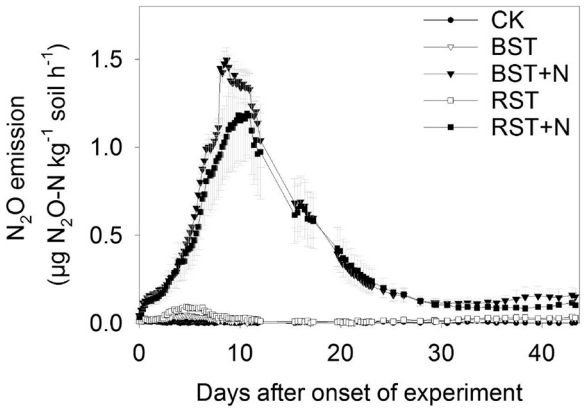

Fig. 2. Daily N_2O emissions from soils after incorporation of oilseed rape straw (RST) and barley straw (BST) without and with N fertilizer (RST + N, BST + N) in the incubation experiment (CK: non-treated control soil). Error bars show the standard error of the mean of each treatment (n = 3). In some cases error bars are smaller than the symbols.

significantly after onset of treatments for about 48 h and remained constant throughout the incubation period ($p < .05$, Fig. 1). Here, maximum rates were 0.2 mg CO_2-C kg^{-1} soil h^{-1} in the control treatment. Addition of straw (RST and BST) induced a significant increase ($p < .05$) in respiration, and maximum CO_2 emissions were reached within five days after onset of treatments (DAO). Afterwards, fluxes of CO_2 decreased steadily but were still significantly higher ($p < .05$) than those of non-amended control soil at 45 DAO (Fig. 1). In RST and BST, maximum CO_2 emissions were 0.6 and 0.5 mg CO_2-C kg^{-1} soil h^{-1}. Overall, daily CO_2 fluxes were almost similar (being slightly lower in RST than in BST) when comparing straw types.

Addition of mineral N to straw amended soils (BST + N and RST + N) decreased CO_2 emissions clearly and the effect was more pronounced in BST treatment. Soil CO_2 emissions in all treatments reached their minimum levels 25 DAO and remained almost constant afterwards.

Overall, cumulative CO_2 fluxes during the 43-day incubation were 402.4 ± 31.9, 402.6 ± 5.8 and 179.9 ± 18.1 mg CO_2-C kg^{-1} soil in BST, RST and control treatments (Table 1). Upon addition of mineral N, cumulative CO_2 emissions were clearly lower in both straw amended soils but the effect was significant only in the barley straw treatment ($p < .05$). Here, the decrease in CO_2 fluxes due to the N supply accounted for 33% in BST whereas in RST it was only 16%. Generally, straw incorporation triggered a release of CO_2 and cumulative CO_2 losses for the entire incubation period may be used to calculate the approximate amount of the added carbon substrate which is mineralized during the incubation. The mineralization of the substrate-C can be estimated as the difference between cumulative CO_2-C evolved in straw-amended soil minus that of the control soil. The calculated share of mineralized straw-derived C was 36%, 14%, 34% and 24% in BST, BST + N, RST, and RST + N treatments respectively.

Table 1
Cumulative CO_2-C and N_2O-N fluxes after 43 days of treatment.

	CO_2 [mg C kg^{-1} soil DM 43 $days^{-1}$]	N_2O[a] [µg N_2O-N kg^{-1} soil DM 43 $days^{-1}$]	Residue-derived ^{15}N-N_2O [µg N_2O-N kg^{-1} soil DM 22 $days^{-1}$]
CK	179.85 (14.81) c	3.25 (0.94) a	n.d.
BST	402.37 (26.03) a	18.61 (1.61) b	0.12 (0.0)
RST	402.64 (4.78) a	26.34 (1.91) b	n.d.
BST + N	264.81 (33.78) bc	438.95 (40.93)	1.48 (0.18)
RST + N	337.36 (8.3) ab	386.71 (74.27)	n.d.

Values are means with ± 1 standard error (n = 3). Different letters within a column indicate significant differences between treatments (Tukey's HSD, $p < .05$).
n.d. = not determined.
[a] Significant differences were calculated for control soils and straw amended soils excluding + treatments.

N_2O emissions remained low until 3 DAO. Then, they increased slightly up to 0.09 and 0.05 µg N_2O-N kg^{-1} soil h^{-1} in RST, and BST treatments, respectively (Fig. 2). Daily N_2O fluxes showed a short-lived peak period from 4 DAO to 7 DAO and in this period they were significantly higher in RST than in BST and CK ($p < .05$). After day 6, N_2O fluxes decreased gradually to the background levels of the non-N fertilized treatments. Addition of N fertilizer led to an immediate and large increase in N_2O fluxes. Fluxes of N_2O in RST + N and BST + N treatments increased almost linearly (up to 1.2 ± 0.2 and 1.5 ± 0.1 µg N_2O-N kg^{-1} soil h^{-1} in RST + N and BST + N treatments) until 10 DAO. Thereafter they showed a continuous and quite rapid decrease and almost reached non-N fertilized background levels within 30 days.

Consequently, cumulative N_2O emissions were significantly lower ($p < .05$) in CK than in both non-N fertilized straw treatments (Table 1). Application of oilseed rape straw (RST) alone induced slightly higher cumulative N_2O emissions compared to CK and BST soils which might be attributed to the lower C/N ratio of the oilseed rape straw. Addition of mineral N (67.5 mg N kg^{-1} soil as CAN) however, led to a strong increase in cumulative N_2O emissions of up to 26-fold. Overall, there were no significant differences in cumulative N_2O emissions between oilseed rape and barley straw amended soils with fertilizer N addition ($p > .05$). Although not being significant ($p > .05$) in the + treatments, the cumulative N_2O emissions were lower in the soils amended with oilseed rape straw than the ones amended with barley straw.

Stable isotope labeling (^{15}N labeled barley straw) was used to study the contribution of the organically bound N in the straw to N_2O emission from straw and straw + N amended soils in BST and BST + N. Overall, the ^{15}N label of the emitted N_2O was very close to the natural ^{15}N abundance indicating dominance of non-straw derived N_2O (Table 1). Overall, the share of barley straw-N derived N_2O emissions was only 1.35% of 0.1 and 0.4% of 1.5 ± 0.18 µg N_2O-N kg^{-1} soil 22 $days^{-1}$ of the cumulative N_2O emissions in BST and BST + N treatments.

3.2. Soil NH_4^+ and NO_3^- dynamics

During the initial phase of the experiment, soil NH_4^+ concentrations were 0.9 ± 0.1, 1.4 ± 0.1, 31.7 ± 5.4, 2.4 ± 0.7 and 29.7 ± 1.0 mg NH_4^+-N kg^{-1} soil in the non-fertilized control soil (CK), BST, BST + N, RST and RST + N, respectively (Fig. 3a). In N fertilized soils, soil NH_4^+ concentrations decreased continuously to background levels within 25 days and remained low (being similar to

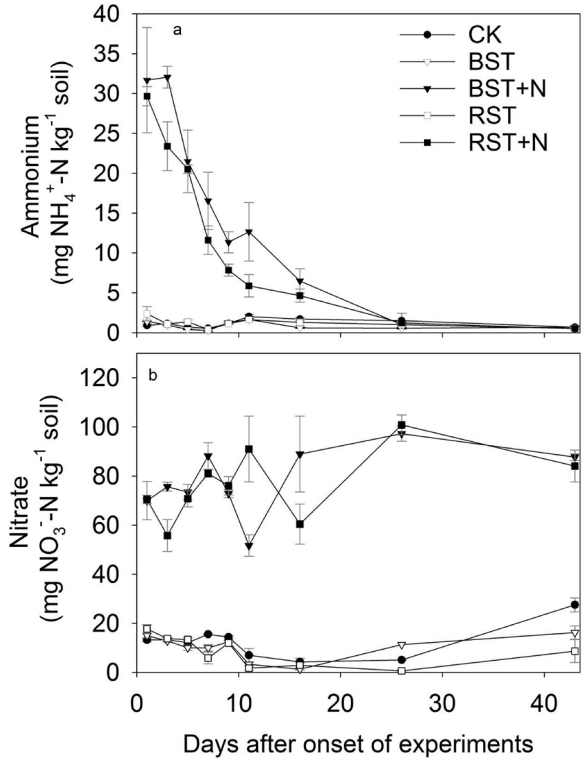

Fig. 3. **a** Soil NH_4^+ content and **b** soil NO_3^- content in soils after incorporation of oilseed rape straw (RST) and barley straw (BST) only and with N fertilizer (RST + N, BST + N) in the incubation experiment (CK: non-treated control soil). Error bars show the standard error of the mean of each treatment (n = 3). In some cases error bars are smaller than the symbols.

CK) afterwards.

Soil NO_3^- concentrations were 13.2 ± 0.8, 15 ± 0.9, 70 ± 7.8, 17.8 ± 1.7 and 70.5 ± 1.7 mg NO_3–N kg^{-1} soil in CK, BST, BST + N, RST and RST + N, respectively, 1 day after onset of treatments (Fig. 3b). In the period of 7–15 DAO, soil NO_3- concentrations in the RST treatments were similar to control soils (CK) and soils amended with barley straw (BST). In N-treated soils (BST + N, RST + N), NO_3- concentrations were always significantly higher than in soils without N supplementation (p = .05). In addition NO_3- concentrations showed a small but continuous increase throughout the experimental period.

3.3. Gene expression of nosZ

The overall average *nosZ* gene expression ranged from 2.1×10^4 to 6.8×10^5 copies per g soil. As shown in Fig. 4 highest *nosZ* copy numbers were observed at day 7 and day 11. Barley straw amended soils (BST) showed highest gene expression at day 11. The highest *nosZ* gene expression with 6.8×10^5 copies per g soil was observed for barley straw and N-fertilized soil (BST + N) at day 7 after onset of treatment, also variation within this treatment was high, ranging from 6.3×10^4 to 1.1×10^6 copies per g soil. Overall the barley straw amended soil (BST) showed the lowest *nosZ* gene expression peak with 1.1×10^5 copies per g soil. The *nosZ* copy numbers at the beginning (1 DAO) and towards the end of the experiment (25 DAO) were on a low and comparable expression level of 2.7×10^4 to 1.1×10^5 copies per g soil, for all treatments.

4. Discussion

4.1. Soil N transformations

The soil NH_4^+ concentrations in the two sole straw treatments

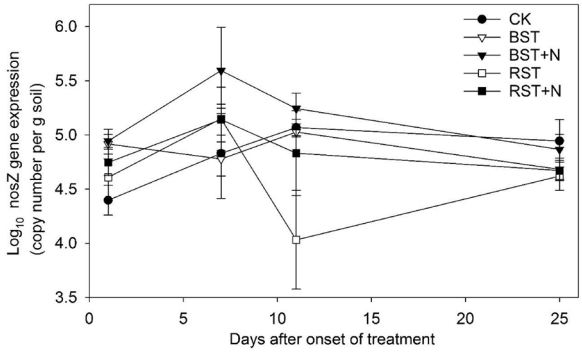

Fig. 4. *nosZ* gene expression at day 1, 7, 11 and 25 from soils after incorporation of oilseed rape straw (RST) and barley straw (BST) without and with N fertilizer (RST + N, BST + N) in the incubation experiment (CK: non-treated control soil). Error bars show the standard error of the median of each treatment (n = 3). In some cases error bars are smaller than the symbols.

remained on a low level and there were no differences among straw types as well as compared to the control soil. There was only a slight decrease until day 7 which might be due to a higher nitrification rate than mineralization of the straw-N in this phase of the experiment. Nitrification is one of the main processes leading to decreasing soil NH_4^+ values (Cai et al., 2001) which was particularly evident in treatments with added N (BST + N, RST + N) where NH_4^+ concentrations showed a very steep decline in the first ten days. Besides nitrification, abiotic immobilization and the preferential uptake of NH_4^+ by soil microorganisms may have contributed to depletion of the soil NH_4^+ concentration (Recous et al., 1990).

The decrease of soil NO_3^- concentration in the sole straw treatments (BST, RST) until day 11 is most likely to be explained by microbial assimilation of N which is boosted by the decomposition activity by the microorganisms (Potthoff et al., 2005). Straw represents an additional carbon source for microorganisms favoring heterotrophic respiration as indicated by increased CO_2 emission rates.

NO_3^- concentrations in + treatments were higher than in non-N-fertilized treatments due to addition of nitrate as CAN. The additional increase until day 27 was probably due to nitrate production in the course of fertilizer ammonium nitrification which is in line with a matching decline in soil NH_4^+ concentrations during this period. As NO_3^- concentrations steadily remained on a high level in + treatments, denitrification obviously played a minor role with respect to soil N balances in the experimental period.

4.2. Effect of straw addition to CO_2 and N_2O emissions

Expectedly, the incorporation of straw into soil led to increased CO_2 emissions. Such rapid degradation of easily accessible C compounds in straw has been shown in earlier studies with similar CO_2 emission patterns and orders of magnitude (Chen et al., 2014c; Geisseler et al., 2009; Huang et al., 2004; Potthoff et al., 2005; Velthof et al., 2002). In our experiment, rather the addition of straw than the quality of straw (C/N) was an important factor for decomposition measured as soil CO_2 emissions. This is supported by Chapin et al. (2002) and Chen et al. (2015) who showed that the addition of a substrate has a much greater influence on the decomposition velocity than its C/N ratio.

N_2O emissions of soils amended with straw only (BST, RST) remained on the background levels during the first three days. It is likely that this lag phase was caused by heterotrophic respiration followed by O_2 consumption (Potthoff et al., 2005). The depletion of O_2 in microsites at the small scale is known to lead to the establishment of microaerophilic or even anaerobic microsites. Consequently, such hot spots provide favorable conditions for denitrification and N_2O production (Parkin, 1987). In BST and RST, N_2O emissions were higher from day 3 to day 7 as compared to control soils (CK) which might be attributed to

the decomposition of incorporated straw-C which enhanced heterotrophic respiration. In a short period of day 3 to 7 emissions in RST were twice as high as in BST which may be attributed to the lower C/N ratio of the oilseed rape straw which was going along with higher availability of organic N. This enhances microbial decomposition/mineralization and the formation of NO_3^- which can lead to denitrification and hence higher N_2O emissions if O_2 is consumed and CO_2 released by mineralization.

These results are supported by several studies where a negative correlation of soil N_2O emissions to the C/N ratio of the soil-incorporated substrate was found (Chen et al., 2013; Frimpong and Baggs, 2010; Huang et al., 2004; Parkin, 1987; Velthof et al., 2002). Though, Chen et al. (2013) explain that an amendment with C-rich residues with a C/N ratio > 45 leads to a high demand of soil N for microbial growth. Here, it can be inferred that the N limitation is aggravated with increasing C/N ratios. In the treatments without mineral N addition, this relationship might have contributed to higher N_2O emissions in the oilseed rape straw amended soils compared to soils amended with the cereal straw of barley.

4.3. The effect of N addition to CO_2 and N_2O emissions

The addition of N and a straw source to soil changes the habitat conditions of soil microorganisms. The soil microorganisms can use the organic matter for energy production as well as metabolic utility of N and modify N cycling. Knorr et al. (2005) showed decreased soil CO_2 release for low-quality litters with high lignin content when in addition mineral N was supplied. In general, it is accepted that the addition of N affects soil C cycling (Knicker, 2011; Liu and Greaver, 2009; Treseder, 2008). Wang et al. (2004) observed that the addition of nitrate to straw and its influence on CO_2 emissions can be separated into two phases. In the early stage of straw degradation, they reported a generally higher rate of C mineralization and higher decomposition activity in the straw + N treatment compared to the sole straw treatment. In the later stages of their experiment, addition of N to straw amended soil reduced the decomposition rate. This was ascribed to a shift in the factors controlling decomposition. With progressing time, the factors moved from N content or C/N ratio in the initial phase to lignin/N and polyphenols/N ratios of the plant material in the treatments without N addition. Interestingly in the + treatments, other characteristics like N-alkyl or methoxyl C showed a correlation to cumulative C mineralization. N-alkyl C is a part of amino acids or sugars and methoxyl C is a substituent of lignin. While lignin/N and polyphenols/N ratios were not analyzed in the present study, the general CO_2 flux dynamics support the findings of Wang et al. (2004), i.e. initially, CO_2 emission rates did not show pronounced effects of N addition while after the first 5–7 days CO_2 fluxes were significantly lower in + treatments as compared to non-N amended equivalents ($p < .05$).

Treseder (2008) described that N addition negatively affects soil characteristics (e.g. a decrease in soil pH) and that microbial biomass may decline significantly with N addition. Thus, a possible reason for the decrease of soil CO_2 emissions in N applied soils may be ascribed to a reduction in microbial biomass and eventually activity like decomposition caused by N fertilization (Treseder, 2008). Nevertheless, the fate of carbon is a point of discussion; it can also be assumed that short-term microbial biomass production after residue addition and thus, uptake of C is enhanced due to the termination of C and N limitation by addition of C and N substrates which leads to the built up of fungal biomass (Bai et al., 2016; Henriksen and Breland, 1999; Rousk and Bååth, 2007).

In contrast to soil treated with straw alone, fertilizer N addition to straw amended soils (BST + N, RST + N) was followed by a steep increase in soil N_2O emissions due to seemingly unlimited NH_4^+ and NO_3^- availability in the first days of the experiment. Nevertheless, the soil NO_3^- pool remained on a stable and high level throughout the experimental period and – without any lag phase - the soil NH_4^+ concentration decreased steadily until day 26. Both, the high concentration of NO_3^- and the continuous decrease in the NH_4^+ pool are seen as evidence that a large share of the NH_4^+ was nitrified in aerobic microsites which probably made a certain contribution to the rapid increase in N_2O emissions. This view is supported by the subsequent period with decreasing N_2O emissions after day 10 since the NO_3^- pool at the subsequent sampling days remained on a high level. Thus, nitrification probably made a certain contribution to soil N_2O emissions; however nitrification has a lower share on N_2O emissions than denitrification (Vilain et al., 2014) as discussed in the following.

Cumulative N_2O emissions increased more than 15-fold when N was added to soils amended with barley or oilseed rape straw as compared to sole straw treatments. N addition to straw amended soils enhanced denitrification and oxidized N compounds such as NO_3^- were used as electron acceptors in heterotrophic respiration. When soil NO_3^- concentrations are high as observed in + treatments, nitrate reduction yields more energy than the reduction of N_2O (Saggar et al., 2013; Weier et al., 1993), thus when NO_3^- availability is high, N_2O reduction is likely to be low, leading to high N_2O emissions and comparatively low emissions of N_2. In addition, only a certain fraction of the soil microbial community is able to reduce N_2O to N_2 (Philippot et al., 2011; Saggar et al., 2013) which strengthens the view that rather N_2O than N_2 is produced in soils with ample N supply.

After approximately 12 days, a decrease in both, N_2O emissions and CO_2 emissions in the N fertilized soils (BST, RST) was observed which suggests that easily degradable carbon compounds were mostly consumed leading to a strong decrease in the activity of the N_2O producing microbial community with a subsequent adaptation to conditions with high N availability but less readily available C in relationship to slower decaying straw compounds.

Furthermore, with respect to the two types of crop residues examined in this study, it is noteworthy that similar to their influence on decomposition, i.e., CO_2 release from soil, the type of straw played a minor role in the formation of N_2O. It can be assumed that the oilseed rape straw, characterized by its slightly lower C/N ratio did not trigger higher N_2O emissions from soil than the straw of the cereal barley. Nevertheless, in agricultural practice the incorporated amount of oilseed rape straw (DM kg^{-1} soil) might be higher (Kaul et al., 1996) as oilseed rape and barley straw can differ in their residue production (Lal, 2005; Lickfett, 1993; Sieling and Kage, 2006).

4.4. *nosZ* expression and microbial metabolism

To examine the potential reduction of N_2O to N_2, we analyzed the transcription of *nosZ* genes using quantitative polymerase chain reaction (qPCR). The transcription of bacterial *nosZ* genes encoding nitrous oxide reductase, was low and ranged on an equal level for all treatments at day 1 and 25 after onset of treatment. This is in accordance with the observed CO_2 and N_2O emissions, which showed the lowest emission rates bevor day 3 and after day 20. At day 1, *nosZ* copy numbers in N fertilized soils (BST + N, RST + N) were low and exponentially increasing values for nitrification-derived N_2O emissions due to quickly decreasing soil NH_4^+ content suggest a low denitrification rate. Nevertheless, the *nosZ* copy number is lowest for control soils (CK) suggesting that the reduction of N_2O to N_2 is also occurring in treated soils (BST, BST + N, RST, RST + N). It could be argued that this minor increase of treated soils compared to control soils may be attributed to the reduction of NO_3^- since soil NO_3^- content is decreasing and NO_3^- reduction is less energy demanding for soil microorganisms than the reduction of N_2O (Saggar et al., 2013). In the sole straw treatments (BST, RST), C-rich substrate can be used as electron donor in the dissimilative metabolism for energy production (anaerobic/heterotrophic respiration). This explanation also supports the decrease in soil NO_3- in BST and RST which might have been used in dissimilative metabolism, i.e. NO_3- reduction. The N addition to barley straw and oilseed rape straw amended soils (BST + N, RST + N) changed habitat conditions in

soil. However, the added N is an easily accessible substrate for soil microorganisms' metabolism and relieves the N limitation. It can be assumed that at a later stage, i.e. 10–14 days after onset of the experiment, a considerable share of the C-rich substrate was immobilized by soil microorganisms together with the N supplied by fertilization. Also no significant differences were observed, a clear peak of *nosZ* gene transcription at day 7 is visible for the barley straw and N-fertilized soil (BST + N), which showed the highest *nosZ* gene transcription. This is also in accordance with the observed CO_2 and N_2O emissions, which had the highest emission rates between day 6 and 10 after onset of treatments. There is a relation between *nosZ* gene expression and high availability of N_2O as a substrate. High production of N_2O reductase leads to decreasing N_2O emissions off soil due to a decreased N_2O/N_2 ratio. Following this argumentation pattern, for the RST + N treatment, we probably missed the highest gene expression of *nosZ*. Also changes in the mRNA transcript abundance should be analyzed cautiously, as they may not reflect protein production rates and activity, due to post transcriptional steps. Contrastingly, the oilseed rape straw treatment with N application (RST + N) showed only a peak of *nosZ* transcription at day 7, while the barley straw (BST) treatment, as well as the control (CK), showed a peak at day 11. The oilseed rape straw amended soils (RST) had the second highest *nosZ* transcription. Oilseed rape straw has compared to barley straw a lower and for microorganisms more favorable C/N than barley straw, as it is closer to the ideal diet (24:1). This leads to higher activity of microorganisms in the oilseed rape straw amended soils (RST) and thus contributes to higher N_2O emissions compared to barley straw. This is also supported by the depletion of the soil NO_3- pool, which in conclusion leads to a demand of other energy sources such as N_2O. The observed higher *nosZ* copy numbers at day 7 in RST treatments probably reflects the change of energy source used and increased demand of N_2O reduction (see Fig. 4).

Overall the variation in the determined transcription rates is high among replicates. The highest variation was observed for the BST + N, ranging from 6.3×10^4 to 1.1×10^6 copies per g soil among the three replicates. Several reasons may have led to the variation among treatments. Generally, there is a time lag between excess N_2O and the *nosZ* gene expression. Bacterial *nosZ* transcription may be enhanced by the N demand of denitrifiers and if the soil NO_3^- pool is depleted soil microorganisms have to seek for an alternative substrate such as the unfavorable N_2O. It is known that the reduction of N_2O is more energy demanding to bacteria (Saggar et al., 2013) and sudden high copy numbers in *nosZ* gene expression may be explained by accumulation of N_2O in microsites. Furthermore, soil heterogeneity may result in varying numbers of microsites causing high deviation in copy numbers within the treatments analyzed.

The high variability of copy numbers within treatments may also be explained by short turnover times of bacterial mRNAs due to RNases and other factors influencing the degradation (Deutscher, 2006).

4.5. Barley straw-N-derived ^{15}N-N_2O

Straw-N-derived N_2O emissions were investigated with barley straw amended soil. N_2O emissions and thus straw-N-N_2O emissions were low in soils amended with barley straw only. N fertilization led to an exponential increase in N_2O emissions and cumulative N_2O emissions were several times higher than in soils amended with barley straw only. Additionally, the share of straw N in N_2O release was even lower for soils amended with barley straw and N fertilizer. A generally low share of organic N-derived N_2O emissions was found in several studies (Frimpong and Baggs, 2010; Gentile et al., 2008; Ocio et al., 1991). In several studies, the fate of straw N was examined after its addition to soil. An alternate pathway of straw N was illustrated in the study of Ocio et al. (1991) which showed that up to $^2/_3$ of the increase in microbial biomass N was attributed to the supply of organic N by straw decomposition. Such direct incorporation of straw N implies that the straw-bound N did not enter the soil mineral N pool. Immobilization of straw N was also observed in an incubation study by Trinsoutrot et al. (2000). Here, the straw addition led to immobilization and in addition, with a higher C input immobilization of N was even higher. This decrease of the soil mineral N pool leaves unfavorable conditions for nitrification or denitrification. Hence as a rule, straw N entering the soil seems to be mostly unavailable for N_2O production. It can therefore be assumed that microorganisms in soils amended with barley straw alone typically undergo N limitation since soil mineral N pools are rapidly depleted. This underlines the possible explanation of direct uptake of organic N by soil microorganisms (Geisseler et al., 2009). Furthermore, the difference in straw-derived N_2O of BST and BST + N may reflect the dominance of different processes in the treatments. In + treatments, it can be assumed that soil N cycling, is accelerated which is clearly shown in the high N_2O emissions. As discussed, the addition of a carbon source and turnover of barley straw N in the + soils may have triggered a quick increase in microbial biomass N, but in parallel, the decline in easily available carbon due to decomposition could have led to remobilization of the biomass-bound N. In a study conducted by Shindo and Nishio (2005), ^{15}N labeled wheat straw was incorporated into soil and nitrate was applied. Here, straw and mineral N led to substantial increase in microbial biomass N after 7 days. At all sampling days, ^{15}N-labeled biomass N was about 10%. In the course of their experiment, there was no evidence, that a share of the straw N steadily increased the NO_3^- pool. Therefore plant residues should be primarily seen as an energy source and when in micro-sites the mineral N pool is exhausted, it will also serve as source of N for microbial biomass production (Chen et al., 2014b). Naturally, soil microorganisms die and the biomass N is released into the soil mineral N pool. Consequently, the formerly straw-bound N may enter the mineral N soil pools where it is available for nitrification, denitrification and eventually release as N_2O or through competitive processes like plant uptake, such as winter wheat following winter oilseed rape.

4.6. Implications for bioenergy production

The presented study was conducted to enlighten the role of post-harvest practices on N_2O emissions. The post-harvest period states a crucial part of the annual CO_2 and N_2O emissions. Here, we tested factors like straw addition which is a common agricultural practice and the effect of a high soil mineral N content. It should be mentioned that the conditions were set to favor denitrification. Firstly, the N rate was unusually high for autumn fertilization and secondly, the moisture level was set to very wet conditions being favorable for denitrifiation. With regard to biofuel crop production the increase of N_2O emissions with the incorporation of straw alone was significant, but nevertheless the magnitude was minor. Under field conditions, incorporation of straw leads to a short-timed peaks in N_2O emissions which may be overestimated on a whole year perspective. Additionally, it is difficult to scale our results to the whole process of biodiesel production as we only considered a short period, the post-harvest, of the total production process which is again divided into growing of crops, fuel production and transport. However, field emissions in the bioethanol/biodiesel production are assumed to have a fixed share (Majer and Oehmichen, 2010).

5. Conclusion

In agriculture, straw incorporation is a common practice in the post-harvest period and this may increase the risk of N_2O emission specifically in moist soils. Our study clearly showed that when soil mineral N content is low, incorporation of barley or oilseed rape straw cause only a slight increase in N_2O emission. However, when soil has high residual N and/or straw amended in conjunction with mineral N, soil N_2O emissions seem to increase drastically. In this context, the present study clearly suggests that straw type seems to have only minor effect on N_2O emission, but soil mineral N content plays a key role. Stable isotope

labelling experiment was in line with that showing only a very small share of the nitrogen emitted as N_2O originates from incorporated organic matter.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/10.1016/j.still.2018.01.013.

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